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PROCESSED PORTULACA
[Kako Suberihiyu]

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SPECIFICATION /191*

1. Title

Processed Portulaca

2. Claims

Whole portulaca plants that are soaked in warm water and subsequently dried for 60 days or longer without exposing them to direct sunlight.

3. Detailed Description of the Invention

The present invention pertains to a product that is obtained by processing portulaca with a unique method and that has an effect that has not been achieved by traditional products.

Portulaca heretofore has been simply treated as a weed, or it has been considered to be a folk medicinal herb and used as an antipyretic/antiphlogistic, antidotal, and detumescent drug or used for relieving pertussis simply by boiling it with water, but it has exhibited only weak effects.

The present invention is characterized by soaking whole portulaca plants in warm water and subsequently drying them for 60 days or longer without exposing them to direct sunlight. Only when portulaca is processed in this manner does it become possible to extract from portulaca an essence that dramatically lowers the blood sugar level of diabetic patients.

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When the extraction liquid obtained from portulaca that was processed according to this unique method was administered to a diabetic patient whose blood sugar level was 220 or higher, the blood sugar level dropped to 150 or thereabouts in fourteen days and became normal. Besides this example, remarkable effects were observed in all of the diabetic patients who took this extraction liquid, although there were some variations in the values.

Thus, the present invention is useful and beneficial for the health of human beings.

Incidentally, with respect to the method for using the processed whole portulaca plants of the present invention, they may be cut into small pieces, stuffed into a tea pack, and boiled with water, thereby obtaining an essence, and this essence is taken. Further, the essence obtained by boiling and extracting the processed portulaca of the present invention with water may be formed into a liquid or a solid and taken in these forms, and doing so yields the same effect.

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Extract of Portulaca oleracea L.
[쇠비름 추출물]

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Nomenclature

Portulaca oleracea, Portulaca oleracea Extract, Functional Drink, Antimicrobial Agent, Anti-carcinogenic Agent

Detailed Description

Detailed Explanation of This Invention

Purpose of This Invention

Technology that This Invention Belongs to and Existing Technology

This invention is about Portulaca oleracea extract that can be used to manufacture drinks which have antimicrobial, anti-carcinogenic, and nicotine eliminating functions and also about the Portulaca oleracea's hot water extract and ethanol extract that can be used to develop antimicrobial or anti-carcinogenic agents.

Portulaca oleracea is an annual coarse grass that belongs to Portulacaceae family and it is also called O Haeng Cho *(O Haeng Cho)*, Jang Myeong Chae *(Jang Myeong Chae)*, Ma Chi Chae *(Ma Chi Chae)*, etc. It grows naturally on the road sides and at a kitchen garden. Its stem is about 15 ~ 30 cm long without hair; its stem is brownish red; and it spreads on the ground because its branch splits into many sections. Its root is white but the color becomes red when the surface is scratched with hands. The leaves grow facing each other or crisscross but the leaf at the tip of branch vertcillates. The leaf looks like a tumbled down egg and it has a short petiole because the tip of the leaf is round and the bottom becomes narrow. The length and width of the leaf is 1.5 ~ 2.5 cm and 0.5 ~ 1.5 cm respectively and its edge is flat. It blossoms between June and October. The flower is yellow and bisexual. The flower is located at the tip of branch and has two oval shaped calyxes. The flower has five petals and opens during the day time briefly

and fades in the evening. It has 7 ~ 1 stamens and one pistil and they are located at the center of an ovary and there are five styles for the flower. Its fruit ripe starting in August and have oval shape. It has many seeds with long culm when the fruit open up at the center. The Portulaca oleracea can be consumed as a food (with seasoning on it) or as an ingredient of medicine. However, the effect and the usage of Portulaca oleracea have been very limited.

As the living standard and the earnings of modern people improves people are more interested about their health and as the result, the development of functional food from natural resources and crude drugs have been conducted actively worldwide and the developments of medicine that has certain functions such as antimicrobial agent or anti-carcinogenic agent have tendency of extracting only the needed elements (medically effective elements) from the raw materials. Above all, the adult diseases such as stomach and/or intestine problems and cancer have been increasing because of irregular diets and busy schedules [of people in modern society] and as the result, the demands of functional foods that can prevent such diseases have been increasing as well. In Korea, various health foods that utilize the extracts from the crude drugs and natural resources have been developed for a long time and the possibility of these health foods preventing these diseases and revitalizing body functions has been presented.

Ginseng, ginger, green tea, pine needles, sagebrush, persimmon, and plum are examples of such foods but these existing functional foods have only one function and have some problems that need to be improved and most importantly they haven't been used to make the functional food that is effective as antimicrobial, anti-carcinogenic, and nicotine removing agent.

Technical Obstacles that This Invention Wants to Overcome

1. Antimicrobial Function (It can be applied in making antimicrobial agent and cosmetics)

In order to study the Portulaca oleracea's growth prohibiting effect on various harmful bacteria, extracts of the Portulaca oleracea (hexane, ethy acetate, chloroform, ethanol, hot water) have been used to examine the antimicrobial effects on Salmonella typhirurium, Sal. paratyphimurium, Staphylococcus aureus, Shigella dysenteriae, Shigella flexneri, Shigella sonnei, Escherichia coli, Pseudomonas aeruginosa.

In order to obtain the MIC (minimum inhibitory concentration), tryptic soy agar medium was used to hold each Portulaca oleracea's extract and the 8 bacteria samples, which was cultivated for 24 hours and was inoculated to the extracts using sterilized swab or platinum loop. These mediums were then cultivated for 18 hours at 37 °C and the bacteria's concentrations had been confirmed.

Base on the result obtained from the solid medium, the concentration that prohibits the bacteria's growth was further developed and the liquid mediums containing the Portulaca oleracea extracts at each concentration were produced. The bacteria samples were inoculated to the mediums and cultivated for 16 ~ 18 hours at 37 °C. The clarity of the liquid medium was measured and the MIC was determined (refer to the table 1).

Among the microorganisms used in the experiment, the salmonella can cause to have infectious disease such as typhoid and the shigella can be the cause of dysentery. The staphylococcus forms enterotoxin causing various food poisoning and skin diseases such as acne. The Escherichia coli, which is used to measure food sanitation standard, and Pseudomonas aeruginosa, which is the most prominent putrefactive bacteria of food, were selected to test the portulaca olearcea extract's antimicrobial capability against these microorganisms to see if the extract can be used as a raw material for antimicrobial agent or functional cosmetics.

As it can be observed from the table 1, the hot water extract, ethanol extract, chloroform extract, and ethyl acetate extract showed strong antimicrobial tendency against testing bacterial samples.

[Table 1]

Portulaca oleracea L Extract's MIC (Minimum Inhibitory Concentration) on Various Harmful Bacteria

Microorganism	Portulaca oleracea extracts				
	Hexane	Ethyl acetate	Chloroform	Ethanol	Hot Water
Salmonella typhirurium	1000	500	500	50	500
Sal. paratyphimurium	1000	500	500	50	500
Staphylococcus aureus	2000	500	500	50	50
Shigella dysenteriae	2000	500	500	500	500
Shigella flexneri	2000	500	500	500	50
Shigella sonnei	2000	500	500	50	500
Escherichia coli	1000	500	500	50	50
Pseudomonas aeruginosa	1000	500	500	50	50

The numerical values in table 1 is expressed the portulaca oleracea's minimum density where the various microorganism's growth was suppressed in ug/ml unit.

As the testing result shows, the portulaca oleracea's hot water, ethanol, ethyl acetate, and chloroform extracts shows strong antimicrobial tendency at 50 ~ 500 mg/ml and can be used as a raw materials of antimicrobial agent and cosmetics for preventing and treating skin disease.

2. Anti-carcinogenic Function

In order to study the Portulaca oleracea extract's effect on various cancer cells' growth, the portulaca oleracea was degreased for 10 hours in hexane and dissolved the degreased portulaca oleracea with ethyl acetate, chloroform, ethanol, and hot water. The extracts of these solvents then used to study the portulaca oleracea extract's anti-carcinogenic effects on stomach cancer cells (SNU-16), colon cancer cells (SNU–C2A), uterine cancer cells (HeLa), and liver cancer cell (SNU-449) and its effects on normal cells. As for the anti-carcinogenic experiment method, 100 μl of medium with various cancer cells and 10% fetal bovine serums was entered in each of 96 well tissue culture plate and they were cultivated at the 5% CO_2 cultivator at 37 °C over night. Then 20 μl of prepared portulaca oleracea extract was inoculated in each well per concentration. Then these wells were cultivated again at the CO_2 cultivator for 48 hours at 37 °C. After that, 50 μl of 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide sample was added and cultivated for 4 hours. 100 μl of DMSO was added in each well and was agitated until formazan is completely dissolved then the absorbance was measured at ELISA reader 540 nm as shown in the table 2.

[Table 2]

Portulaca oleracea Extract's Carcinostatic Effect on Various Cancer Cells

Extract of Portulaca oleracea (Added Density: ug/ml)	Absorbance Measured in Various Cells				
	Stomach Cancer Cells	Colon Cancer Cells	Uterine Cancer Cells	Liver Cancer Cells	Normal Cells
Ethylacetate Extracts 0	1.463	1.305	1.231	1.325	1.034
Ethylacetate Extracts 50	0.657	0.736	0.582	0.678	1.179
Ethylacetate Extracts 500	0.456	0.478	0.431	0.485	1.132
Ethylacetate Extracts 5000	0.431	0.369	0.419	0.411	1.197
Chloroform Extracts 0	1.359	1.463	1.394	1.217	1.099
Chloroform Extracts 50	0.738	0.638	0.601	0.598	1.101
Chloroform Extracts 500	0.412	0.321	0.343	0.495	1.062
Chloroform Extracts 5000	0.465	0.437	0.392	0.438	1.117
Ethanol Extracts 0	1.375	1.328	1.409	1.399	1.205
Ethanol Extracts 50	1.109	1.023	0.948	0.996	1.119
Ethanol Extracts 500	0.476	0.448	0.378	0.401	1.254
Ethanol Extracts 5000	0.465	0.472	0.402	0.407	1.185
Hot Water Extracts 0	1.297	1.309	1.386	1.359	1.128
Hot Water Extracts 50	0.906	0.898	0.921	0.798	1.109
Hot Water Extracts 500	0.347	0.302	0.385	0.296	1.163
Hot Water Extracts 5000	0.401	0.327	0.407	0.305	1.174

The measured absorbance is proportional to the number of live cells in the well.

This means that the ethylacetate, chloroform, ethanol, and hot water extracts of portulaca oleracea had no influence on the normal cells as shown in the table 2 but they showed strong anti-carcinogenic tendency (50% or more) at 500 ug/ml or more concentrations on stomach cancer, colon cancer, uterine cancer, and liver cancers. This result show that the portulaca oleracea extracts can be used as an anti-carcinogenic agent.

And the portulaca oleracea extracts from this invention can be used agricultural chemical as well.

In order to find out the application of the portulaca oleracea extracts as a food preservative or as agricultural chemicals which prevents damages by blights and harmful insects on fruits and vegetables, the extract's anti-fungals against fungus from fruits and vegetables were examined. The growth inhibition effects against fungus from strawberry, Glomerella ciugulata from apple, Rhizopus agar (PDA) from sweep potato were examined. As for the growth inhibition experiment method, potato dextrose agar (PDA) medium and the portulaca oleracea extracts in each concentration were placed on Petri dish and solidified. The solidified fungus was cut in a circle with 6 mm diameter and placed at the center of the dish then cultivated for 3 days at the cultivator at 25 °C. After the cultivation, the diameters of the cultivated fungus were measured and the measurement was compared with the comparing fungus group which was cultivated without the portulaca oleracea extracts. The table 3 shows that the portulaca oleracea extracts (ethyl acetate, chloroform, ethanol, hot water extracts) have strong anti-fungals against the separated fungus.

[Table 3]

Portula oleracea Extract's Growth Inhibitory Effect on Various Harmful Fungus

Types of Portula oleracea Extract (Density: 1000 ppm)	Type of Various Fungus (Diameter of Growth: mm)			
	Tangerine	Strawberry	Apple	Sweet Potato
Comparing Group	87 mm	92 mm	91 mm	93 mm
Ethyl Acetate Extract	15 mm	13 mm	14 mm	13 mm
Chloroform Extract	14 mm	14 mm	13 mm	12 mm
Ethanol Extract	15 mm	15 mm	14 mm	13 mm
Hot Water Extract	15 mm	14 mm	12 mm	12 mm

But, the comparing group inoculated the PDA medium with the same fungus at the same condition with the same size without the portulaca oleracea.

As the results from above show, the ethyl acetate, chloroform, ethanol, hot water extract has more than 80% inhibiting effect on various funguses.

3. Nicotine Removing Function

And the portulaca oleracea extract's nicotine, which is one of the harmful elements of cigarette, removing function was examined as in the following.

The same amount of portulaca oleracea extract solvents and the nicotine from 20 different cigarettes were mixed and left the mixture 5 hours at 37 °C for their reaction. After that, the mixtures were centrifugally separated and the upper layer was analyzed using HPLC method. The analysis results of nicotine content using the peak area of HPLC chromatogram show that the portulaca oleracea hot water extract has 89% nicotine removing effects compare to the comparing group, which does not have any extracts, as shown from the table 4.

[Table 4]

Portula oleracea Extract's Nicotine Elimination Effect

Sample	Nicotine Content among Tobacco Extracts (peak area)	Elimination Ratio (%)
Tobacco Extracts + Comparing Group	273184913314581	10 (base area)
Tobacco Extracts + Portulaca oleracea hexane Extracts	981637121585122	51
Tobacco Extracts + Portulaca oleracea ethylacetate Extracts	9332279949	27
Tobacco Extracts + Portulaca oleracea chloroform Extracts		55
Tobacco Extracts + Portulaca oleracea methanol Extracts		55
Tobacco Extracts + Portulaca oleracea distilled water Extracts		89

The same amounts of the nicotine extracts and methanol, which is HPLC solvent, instead of portulaca oleracea extract were mixed at the comparing group.

4. Functional Drink

Also, in order to provide a functional drink that uses the portulaca oleracea extract as its ingredient, the functional drink manufacturing procedures were described in this invention.

Portulaca oleracea L. can be obtained easily, it is inexpensive, and it is an annual coarse grass that belongs to Portulacaceae family. This invention uses the portulaca oleracea as a main

ingredient of the functional drink. The portulaca oleracea has 0.25% of I-Nor adrenalin ($C_8H_{11}O_3N$), the same amount of dopamine ($C_8H_{11}O_2N$), and small amount of dopa ($C_9H_{11}O_4N$). It also has other elements such as KCl (in forms of nitrate, bupropriom, and lactate and it contains 1% in regular form and 10 ~ 17% in dry form when calculating with K_2O method), large amount of organic acid (malic acid and citric acid), amino acid (glutamine acid, aspartic acid, alanine), cardio glycoside, antrakinon glycoside (TC - transliterated), etc. And the fresh grass contains 94 ~ 96% of water and the dry grass has 4.5 ~ 6.6%. The dried grass has 14 ~ 24% of crude protain, 3.7 ~ 5.8% of crude fat, 25 ~ 27% of crude ash, 2 ~ 3% of monosaccharide, 4.4 ~ 6% of sugar, 9 ~ 14% of polysaccharide (which is soluble in water), vitamin B_1 and C, 9.4 ~ 17 mg% of carotene, 25 ~ 50 mg% of nicotinic acid, and light weight elements such as zinc, copper, magnesium, nickel, iron, saponin, and tannin, etc. The portulaca oleracea, which is composed of the above elements, are placed in hexane for 10 hours to remove fat completely and the degreased portulaca oleracea residues are solved in ethyl acetate, chloroform, or ethanol solvents. The solved and extracted portulaca oleracea extracts are filtered so that its purity can be 99.9% and process them in forms of liquid or powder. Here, the portulaca oleracea hot water extract obtained in forms of liquid or powder by heating for one hour at 90 °C under high pressure with pressurized pot and then by filtering. The portulaca oleracea extract, which is obtained by using the above method, are used to produce drinks following the applications below.

(Application 1)

Mix the portulaca oleracea hot water extract, cinnamon hot water extract, and ginger hot water extract in 3:1:1 ratio and make a drink.

(Application 2)

Mix the portulaca oleracea hot water extract, licorice, ginger, jujube hot water extracts, and peppermint hot water extract in 15:4:1:1:0.5 ratio and make a drink.

(Application 3)

Mix the same amount of portulaca oleracea hot water extract and cinnoman hot water extract and add 7% of sugar, 0.1% of mono fatty acid glycerin and make a drink.

Here, the portulaca oleracea or cinnamon, ginger, licorice hot water extracts are made by heating for one hour at 90 °C with a pressurized pot; and by squeezing the heated ingredients with a squeezer; and by sterilizing the squeezed liquid for 20 min at 70 °C.

The produced functional drinks, following above applications, were tested by 10 taste testers. For the taste test, ranking test method was used so that the three portulaca oleracea drinks were placed in front of the testers with three digit numbers and the testes were provided with testing paper. Table 5 shows the content of testing paper and the direction asks the tester to taste the drinks from left to right and rank the drinks in order of preference (mark 1 for the most favorite drink and 4 for the least favorite). As the result, the drink from the application 2 was the most favorite drink and the results of antimicrobial and anti-carcinogenic experiments of the drinks are shown on the table 6 and 7. The application 2 had anti-carcinogenic and antimicrobial effects.

[Table 5]

* Taste Test		
Name _____ Date _____ Please rank the taste of the Protulaca oleracea drinks from left to right (most favorite 1, least favorite 4) 1. _____ 2. _____ 3. _____ 4. _____ Opinion:		
* Test Result		
Testing Sample	Combined Point	Ranking
Protulaca oleracea Drink Application 1	21	2
Protulaca oleracea Drink Application 2	11	1
Protulaca oleracea Drink Application 3	28	3

Ranking test and the test result for the taste test

The portulaca oleracea drinks were placed at a rotary vacuum condenser and the condensed drink was dissolved in 70% ethanol so that the density becomes 0.1 g/ml in order to study its effects on the uterine cancer cells (HeLa), stomach cancer cells (SNU-16), colon cancer cells (SNU-C2A), liver cancer cells (SNU-449), and on the fibroblast.

As for the anti-carcinogenic experiment method, 100 μl of medium with various cancer cells and 10% fetal bovine serums was entered in each of 96 well tissue culture plate and they were cultivated at the 5% CO_2 cultivator at 37 °C over night. Then 20 μl of prepared portulaca oleracea extract was inoculated in each well. Then these wells were cultivated again at the CO_2 cultivator for 48 hours at 37 °C. After that, 50 μl of 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide sample was added and cultivated for 4 hours. 100 μl of DMSO was added in each well and was agitated until formazan is completely dissolved then the absorbance was measured at ELISA reader 540 nm as shown in the table 6.

[Table 6]

Carcinostatic Test Result of Portulaca oleracea Drinks

Extract of Portulaca oleracea (Added Density: ug/ml)	Absorbance Measured in Various Cells				
	Stomach Cancer Cells	Colon Cancer Cells	Uterine Cancer Cells	Liver Cancer Cells	Normal Cells (Fibroblast)
Portulaca oleracea Drink Application 1	1.149	1.138	1.362	1.397	1.193
Portulaca oleracea Drink Application 2	0.562	0.476	0.501	0.498	1.105
Portulaca oleracea Drink Application 3	1.256	1.426	1.354	1.327	1.038

The measured absorbance is proportional to the number of live cells in the well.

This means that all three portulaca oleracea drinks had no influence on the normal cells as shown in the table 6 but the drink from the application 2 showed strong anti-carcinogenic tendency

(50% or more). This result showed that the portulaca oleracea drink has an anti-carcinogenic function.

As for the antimicrobial experiment method, The portulaca oleracea drinks were placed at a rotary vacuum condenser and the condensed drink was dissolved in 70% ethanol so that the density becomes 0.1 g/ml. 1 ml of the dissolved drink was taken and was mixed with 10 ml of Tryptic Soy Agar medium and the mixture was solidified on a Petri dish. Here, the 8 bacteria samples, which were cultivated for 24 hours, were inoculated to the extracts using sterilized swab or platinum loop. These mediums were then cultivated for 18 hours at 37 °C and the bacteria's concentrations had been confirmed. The table 7 shows the result of this experiment.

[Table 7]

Microorganism	Types of Portulaca oleracea Drink		
	Application 1	Application 2	Application 3
Salmonella typhirurium	O	X	O
Sal. paratyphimurium	O	X	O
Staphylococcus aureus	O	X	O
Shigella dysenteriae	O	X	O
Shigella flexneri	O	X	O
Shigella sonnei	O	X	O
Escherichia coli	O	X	O
Pseudomonas aeruginosa	O	X	O

Here, "O" means the microorganism grew and "X" means the microorganism did not grow.

The Effect of the Experiment

As it was discussed in detail, this invention obtains the extract of portulaca oleracea, which can be obtained easily, using hexane, ethanol, hot water solvents; obtains high dense portulaca oleracea extracts using various purifying and filtering processes; and produces functional drinks by mixing the extracts with various drinks in certain ratio so that it can provide the portulaca oleracea's functions during the food consumption. On the top of that, this invention can uses the portulaca oleracea extracts for the developments of agricultural chemicals, cosmetics, antimicrobial agent, nicotine removing agent, preservative, and anti-carcinogenic agent.

(57) Range of Claim

Claim 1

The normal portulaca oleracea are heated with hot water; and extracted by squeezing; and the portulaca oleracea extracts are processed in a liquid or a powder form.

Claim 2

From the claim 1;

The above portulaca oleracea was dissolved in an ethanol solvent; and extracted by filtering; and the portulaca oleracea extracts are processed in a liquid or a powder form.